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Multiple independent interspecies transmissions explain the high genetic diversity in three bovine-like human G8P[14] and G10P[14] rotaviruses

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Multiple independent interspecies transmissions explain the high genetic diversity in three bovine-like human G8P[14] and G10P[14] rotaviruses

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Abstract

The group A rotavirus (RVA) P[14] genotype has been sporadically detected in humans and is thought to be acquired through zoonotic transmission. The present study describes the full-length genome analysis of two G8P[14] and one G10P[14] human RVAs detected in Italy. The strains possessed the typical bovine-like I2-R2-C2-M2-A3/A11-N2-T6-E2-H3 genotype constellation. All the segments of the two G8P[14] RVAs were most closely related to bovine (-like) strains but were relatively distant to each other suggesting two independent interspecies transmission events. Likewise, the G10P[14] RVA gene segments were most similar to bovine (-like) RVAs, but distinct from the G8 strains. The natural history of these strains probably involved the interspecies transmission of these viruses to humans from a yet unidentified animal host, without evidence of reassortment events involving human RVAs. These results reinforce the potential of animal viruses to cross the host-species barrier causing disease and increase viral genetic diversity in humans.

Group A rotavirus (RVA), a member of the genus *Rotavirus*, in the *Reoviridae* family, is the leading etiological agent of severe gastroenteritis in the young of humans and many animal species worldwide [Estes & Greenberg, 2013; Martella et al., 2010].

The RVA genome is protected by the viral inner core, and is made up of 11 segments of double-stranded RNA, which encode six structural viral proteins (VP1–VP4, VP6, and VP7) and five or six non-structural proteins (NSP1–NSP5 and sometimes NSP6) [Estes & Greenberg, 2013]. Because of the segmented nature of the genome, reassortment between and among human and animal strains is one of the major processes of genetic evolution of rotaviruses.

The traditional binomial RVA classification system was based on the two outer capsid proteins, VP7 and VP4 and at least 27 G-types and 37 P-types, respectively, have been identified [Matthijnssens et al., 2011; Trojnar et al., 2013]. Extending this classical binomial genotyping system, a new RVA classification system was proposed in which all 11 segments are considered [Matthijnssens et al., 2011]. Following this new classification system, the notations of Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx are used for the VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5/6 encoding genes, respectively. Whole-genome sequencing has become widely established in RVA research, because it offers a platform providing a better insight into RVA diversity and evolution.

In human RVAs, G1, G2, G3, G4, G9, and G12 combined with P[4], P[6], and P[8] are frequently detected throughout the world, [Bányai et al., 2012; Gentsch et al., 2005; Patel et al., 2011; Santos & Hoshino, 2005]. Together with these typical human G- or P-genotypes, a plethora of uncommon genotypes have also been identified in humans such us G6P[14], G8P[14], and G10P[14] [Bányai et al., 2009; Bányai et al., 2010; Matthijnssens et al., 2009a]. The detection of uncommon RVA genotypes in humans has been linked to the frequent intersections between the evolutions of human and animal RVAs, as a result of numerous interspecies transmission events sometimes accompanied by reassortment or adaptation to the new host [Degiuseppe et al., 2013;

74 Martella et al., 2010; Weinberg et al., 2012]. G6, G8 and G10 are the most common RVA G-
75 genotypes encountered in cattle [Bányai et al., 2003; Matthijnssens et al., 2008; Papp et al., 2013].
76 The P[14] RVA genotype has been sporadically detected in humans and is thought to be acquired
77 through zoonotic transmission as it has been found in RVA strains from several distinct animal
78 species, such as rabbits, cows, goats, sheep, and guanaco. P[14] strains are most commonly found in
79 combination with G3 or the typical bovine-like G-genotypes: G6, G8 and to a lesser extent G10
80 [Chitambar et al., 2011; Esona et al., 2009; Okada & Matsumoto, 2002; Parreno et al., 2004].
81 In the area of Parma, Northern Italy, continual uninterrupted hospital-based surveillance for RVA
82 infection has been conducted in the last 27 years [Medici et al., 2007; Medici et al., 2014]. RVA
83 genotyping data were generated during a two-year molecular epidemiological survey in 2004-2005
84 and from 2008 onwards, by sequence characterization of the VP7 and VP4 gene segments. Three
85 unusual RVA strains were found, including one G10P[14] strain, RVA/Human-
86 wt/ITA/PR457/2009/G10P[14] (PR457/2009), and two G8P[14] strains, RVA/Human-
87 wt/ITA/PR1300/2004/G8P[14] (PR1300/2004) and RVA/Human-wt/ITA/PR1973/2009/G8P[14]
88 (PR1973/2009). Preliminary sequence and phylogenetic analyses of the partial VP6, VP4 and VP7
89 genes of the P[14] RVA strain PR1300/2004 showed that this strain may have been of animal origin
90 [Medici et al., 2008].
91
92 In the present study we analysed the full genome of two G8P[14] and one G10P[14] RVAs detected
93 in the area of Parma during an epidemiological survey in order to obtain conclusive data on the
94 overall genetic makeup and to elucidate the origin of these rare human RVA strains. RVA strain
95 PR1300/2004 (G8P[14]) was detected in the stool of a five-year old child in 2004, PR1973/2009
96 (G8P[14]) in an eight-month old child in 2009 and PR457/2009 (G10P[14]) in a one-year old child
97 in 2009. The children were admitted with acute gastroenteritis at the University Hospital of Parma
98 during the epidemic period (December-April). All the 11 gene segments of the Italian RVA strains

were submitted to RT-PCR, Sanger sequencing and phylogenetic analysis. Briefly, the RVA RNA was subjected to RT-PCR (OneStep RT-PCR kit, Qiagen, Westburg, The Netherlands), using primers for all 11 RNA segments as described previously [Matthijnssens et al., 2008]. After visualization in ethidium bromide-stained 6% PAGE gels, PCR products were purified and sequenced. Primer walking was used for full-length sequencing of longer genes (VP1-VP4, and NSP1). Sequences were corrected with ChromasPro2.23 (Technelysium, Queensland, Australia). Nucleotide sequence similarity searches were performed using the BLAST server on the NCBI GenBank database. Multiple alignments were made for each gene segment together with appropriate reference sequences available in GenBank. The sequences included the human reference strains Wa, DS-1 and AU-1, and RVA strains with G/P-genotype combinations equal to those of the analyzed Italian strains. Multiple sequence alignments and phylogenetic tree constructions were performed with MEGA 6 [Tamura et al., 2013], applying the maximum-likelihood (ML) method. GenBank accession numbers (VP7, VP4, VP6, VP1-VP3, NSP1-NSP5) for each individual genomic segment of the three Italian P[14] strains are: KP198625-KP198635 (PR457/2009), KP198636-KP198646 (PR1300/2004) and KP198647-KP198657 (PR1973/2009).

In total, 11 phylogenetic trees were built using the nucleotide sequence of the 11 ORFs of PR457/2009, PR1300/2004, and PR1973/2009. The obtained nomenclature and genotype constellations of the three RVA strains are provided in Table I and the phylogenetic trees are shown in Figure 1.

The Italian RVA strains PR1300/2004 and PR1973/2009 were found to have an identical genotype constellation, G8-P[14]-I2-R2-C2-M2-A3-N2-T6-E2-H3, and were most similar to the human RVA strain RVA/Human-wt/HUN/BP1062/2004/G8P[14] detected in Hungary [Bányai et al., 2010] and the ovine strain RVA/Sheep-tc/ESP/OVR762/2002/G8P[14] detected in Spain [Matthijnssens et al., 2008], sharing the same genotype constellation except for NSP1 (A3 *vs.* A11), and to a guanaco

RVA strain (RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14]) [Matthijnssens et al., 2009a], sharing the same genotype constellation except for VP1, NSP3 and NSP4 (Table I).

The Italian RVA PR457/2009 possessed the G10-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genotype constellation, which is an identical genotype constellation as was found in RVA/Human-wt/AUS/V585/2011/G10P[14]) [Cowley et al., 2013]. The only other completely sequenced G10P[14] strain (RVA/Human-tc/GBR/A64/1987/G10P10[P14]) [Heiman et al., 2008] showed different genotypes for VP3 (M2 *vs.* M1) and NSP1 (A11 *vs.* A3) when compared to strain PR457/2009.

In the phylogenetic tree of VP7, the two Italian G8P[14] RVAs (PR1300/2004 and PR1973/2009) segregated in the same subcluster of the G8 genotype with a 2% nucleotide difference among each other. Both strains had a very close relationship with artiodactyl and artiodactyl-like human strains (similarity up to 98% on the nt level) segregating closely with two porcine/bovine reassortant RVA strains detected in Korea (RVA/Porcine-wt/KOR/07-109-8/2007/G8P[7] and RVA/Porcine-wt/KOR/174-1/2006/G8P[7] [Kim et al., 2010]), two guanaco RVA strains detected in Argentina (RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] and RVA/Guanaco-wt/ARG/RioNegro/1998/G8P[1] [Matthijnssens et al., 2009a]), and slightly more distantly related to a human strain isolated in Kenya (RVA/Human-wt/KEN/B12/1997/G8P[14]), which also has an artiodactyl origin [Ghosh et al., 2011].The analyses of the VP1-VP4, VP6, NSP2-NSP5 gene segments showed that both G8 strains clustered together with bovine and bovine-like G6P[14] and G8P[14] RVAs, but in contrast with the phylogenetic tree of VP7, PR1300/2004 and PR1973/2009 were only distantly related to each other for the other gene segments with exception of NSP5, suggesting that both strains are the result of two independent interspecies transmission events.

In the phylogenetic tree of VP7, the Italian G10P[14] RVA strain (PR457/2009) was most similar to equine, porcine, and bovine RVA strains (96.4-99%), while the most closely related human RVA strain was an old unusual bovine-like human strain (A64), showing only 87.3% similarity on the

149 nucleotide level. For the VP4, VP6 and NSP1-NSP5 gene segments, PR457/2009 was
150 phylogenetically closely related with bovine-like strains, showing nucleotide identities ranging from
151 95.6% to 99.8%. The VP2 segment was most closely related with two goat RVA strains,
152 RVA/Goat-tc/BGD/GO34/1999/G6P[1] and RVA/Goat-xx/CHN/XL/2010/G10P[15], and two
153 human bovine reassortant RVA strains, RVA/Cow-wt/GHA/GH018-08/2008/G8P[6] and
154 RVA/Cow-wt/GHA/GH019-08/2008/G8P[6], recently detected in Ghana [Dennis et al., 2014]. For
155 VP1 and VP3, PR457/2009 showed a close clustering with, RVA/Human-
156 wt/ITA/PAI11/1996/G2P[4] and RVA/Human-tc/SWE/1076/1983/G2P[6], respectively, which
157 were reported to have a bovine-like VP1 and VP3 in a human RVA genetic backbone [Ghosh et al.,
158 2013; Giammanco et al., 2014]. In addition, the PR457/2009 VP3 segment clustered closely to
159 another bovine-like strain, RVA/Human-wt/HUN/BP1062/2004/G8P[14], identified during a
160 surveillance study in Hungary [Bányai et al., 2010].
161
162 The three Italian P[14] RVA strains analysed possessed the typical bovine-like I2-R2-C2-M2-
163 A3/A11-N2-T6-E2-H3 genotype constellation. They clustered together with animal RVA strains or
164 human RVA strains believed to be of (partial) artiodactyl origin. Our data strongly suggest that
165 these three P[14] strains are the result of independent zoonotic transmissions from an animal
166 belonging to the artiodactyla order to a human, as has been described on multiple occasions in the
167 past [Bányai et al., 2009; Cowley et al., 2013; Donato et al., 2014; El Sherif et al., 2011; Ghosh et
168 al., 2007; Matthijnssens et al., 2009a; Mullick et al., 2012]. To the best of our knowledge this is the
169 first report of G8P[14] and G10P[14] bovine-like RVAs in Italy as well as one of few whole
170 genome based characterization of these rare RVA strains worldwide. To date, in Italy P[14] RVAs
171 were reported sporadically, such as in Sicily during 1987–1988, and in 2003 [De Grazia et al., 2011;
172 De Grazia et al., 2014] in conjunction with the G6 genotype (bovine-like). G8 and G10 strains were
173 found exclusively during 2008-2009 in different Italian territories, associated with P[4] or P[8],

without showing evidence of zoonotic reassortment events [De Grazia et al., 2014; Ianiro et al., 2014; Ruggeri et al., 2011]. Furthermore, G6, G8 and G10 RVAs are common in bovines and buffaloes in Southern Italy [Pisanelli et al., 2005].

Full-length genome sequencing of G6 strains has revealed a close genetic relatedness between human G6P[14] and animal G6P[14] and G8P[14] viruses, reinforces that animal RVAs impact on the evolution of human RVAs [Banyai et al., 2010; De Grazia et al., 2011; Matthijnssens et al., 2009b]. The accumulation of whole genome sequencing data will provide new insights into the mechanisms driving the evolution of RVAs.

It is currently unclear why interspecies transmitted strains are unable to further spread in the human population. Some recent findings indicated that the VP8* region of the P[14] VP4 protein interacts with the type A histoblood group antigens of humans [Hu et al., 2012; Liu et al., 2012] as well as bovine and porcine mucins [Liu et al., 2012] and this is thought to play a role in cross-species transmission of P[14] RVAs. This study reemphasizes that humans are susceptible to infection with bovine-like RVA strains, but the further efficient spread of these viruses in the human population is likely prevented by other unknown factors. Continued surveillance of RVA strains will be essential to determine the extent of similar strains in the population in the context of whether or not vaccine-induced heterotypic immunity is sufficient to protect against strains that express the P[14] RVA genotype.

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Figure 1.

Phylogenetic dendrograms based on the nucleotide sequences of all 11 RVA gene segments. Italian G8P[14] strains are marked with a filled circle and completely sequenced G8P[14] strains are indicated with a empty circle. The Italian G10P[14] strain is marked with a filled triangle and other completely sequenced G10P[14] strains are indicated with an empty triangle. Trees were built with the maximum-likelihood (ML) method, and bootstrapped with 1000 repetitions. Bootstrap values >70% are indicated. The scale bar indicates the number of nucleotide substitutions per site.

Table
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Table I. Comparison of the genotype constellation of Italian P[14] RVAs with selected animal RVAs, human bovine-like RVAs and human reference RVAs.

	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5	Shared genotypes
RVA/Human-wt/ITA/PR1300/2004/G8P[14]	**G8**	**P[14]**	**I2**	**R2**	**C2**	**M2**	**A3**	**N2**	**T6**	**E2**	**H3**	
RVA/Human-wt/ITA/PR1973/2009/G8P[14]	**G8**	**P[14]**	**I2**	**R2**	**C2**	**M2**	**A3**	**N2**	**T6**	**E2**	**H3**	
RVA/Human-wt/HUN/BP1062/2004/G8P[14]	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14]	G8	P[14]	I2	R5	C2	M2	A11	N2	T6	E12	H3	8
RVA/Macaque-tc/USA/PTRV/1990/G8P[1]	G8	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3	10
RVA/Human-tc/KEN/B12/1987/G8P[1]	G8	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3	10
RVA/Goat-wt/ARG/0040/2011/G8P[1]	G8	P[8]	I2	R5	C2	M2	A3	N2	T6	E12	H3	8
RVA/Guanaco-wt/ARG/Rio_Negro/1998/G8P[1]	G8	P[1]	I2	R5	C2	M2	A13	N2	T6	E12	H3	7
RVA/Antelope-wt/ZAF/RC-18/2008/G6P[14]	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	9
RVA/Human-wt/HUN/BP1879/2003/G6P[14]	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	9
RVA/Human-tc/ITA/PA169/1988/G6P[14]	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3	10
RVA/Human-wt/ITA/PR457/2009/G10P[14]	**G10**	**P[14]**	**I2**	**R2**	**C2**	**M2**	**A11**	**N2**	**T6**	**E2**	**H3**	
RVA/Human-wt/AUS/V585/2011/G10P[14]	G10	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	11
RVA/Human-tc/GBR/A64/1987/G10P11[14]	G10	P[14]	I2	R2	C2	M1	A3	N2	T1	E2	H3	8
RVA/Antelope-wt/ZAF/RC-18/2008/G6P[14]	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Human-wt/HUN/BP1879/2003/G6P[14]	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	10
RVA/Human-tc/ITA/PA169/1988/G6P[14]	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3	9
RVA/Cow-tc/CHN/DQ-75/2008/G10P[11]	G10	P[11]	I2	R2	C2	M2	A3	N2	T6	E2	H3	9
RVA/Buffalo-xx/ZAF/Buf1442 07SA/2007/G10P[11]	G10	P[11]	I2	R2	C2	M2	A13	N2	T6	E2	H3	9
RVA/Sheep-xx/CHN/CC0812-1/2008/G10P[15]	G10	P[15]	I10	R2	C2	M2	A11	N2	T6	E2	H3	9
RVA/Sheep-tc/CHN/Lamb-NT/XXXX/G10P[15]	G10	P[15]	I10	R2	C2	M2	A11	N2	T6	E2	H3	9
RVA/Human-wt/IND/N155/2003/G10P[11]	G10	P[11]	I2	R2	C2	M2	A1	N1	T1	E2	H3	7
RVA/Human-tc/USA/Wa/1974/G1P[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1	0
RVA/Human-tc/USA/DS-1/1976/G2P[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2	6
RVA/Human-tc/JPN/AU-1/1982/G3[9]	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3	1

Figure

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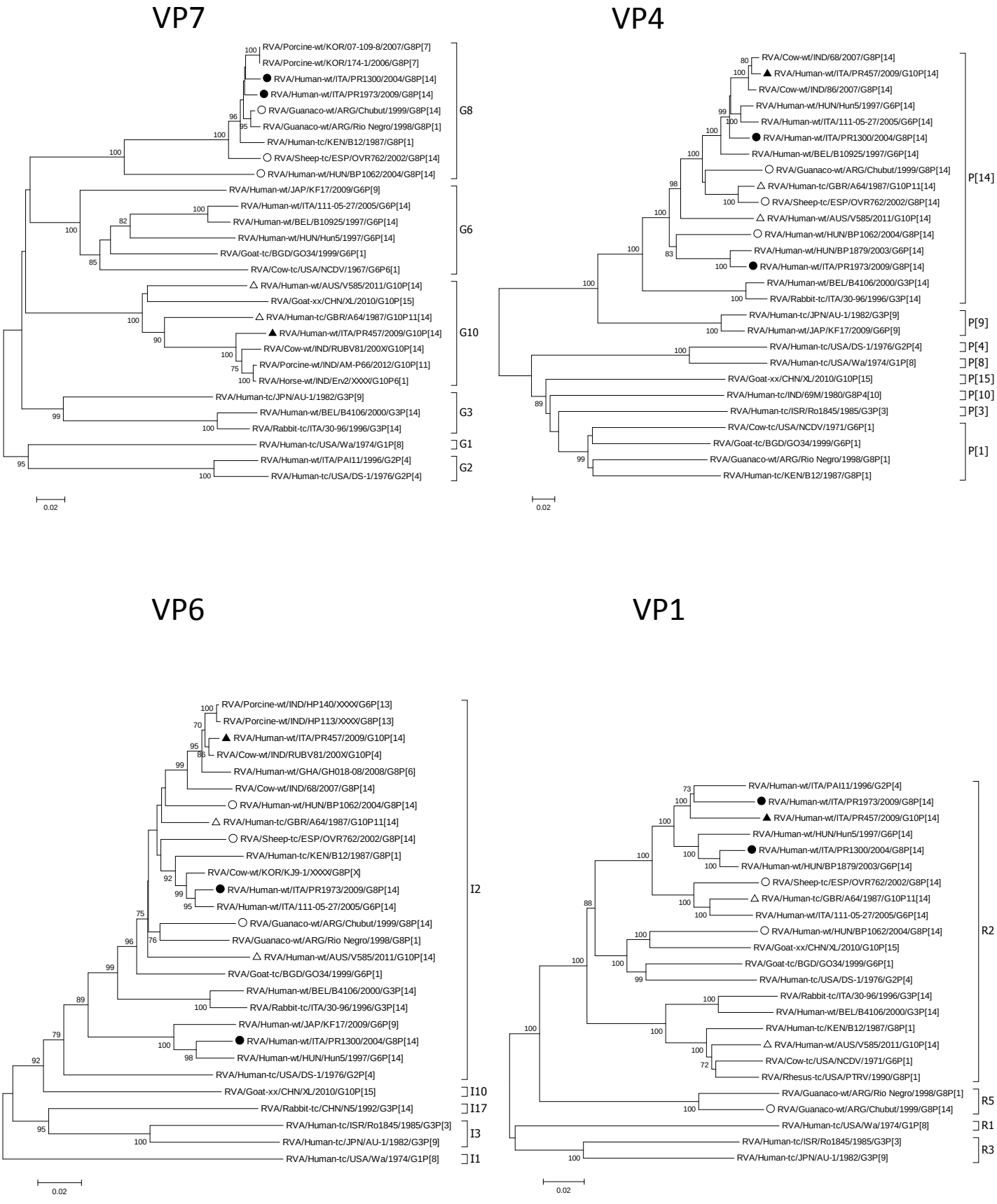

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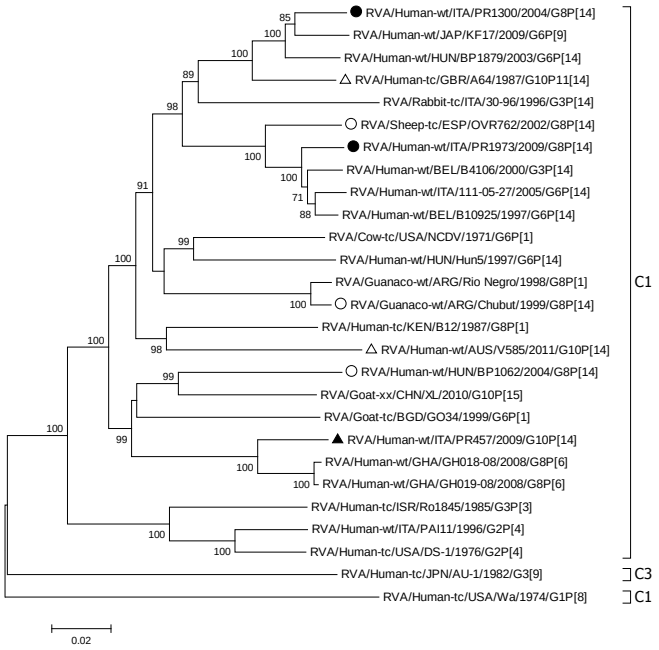

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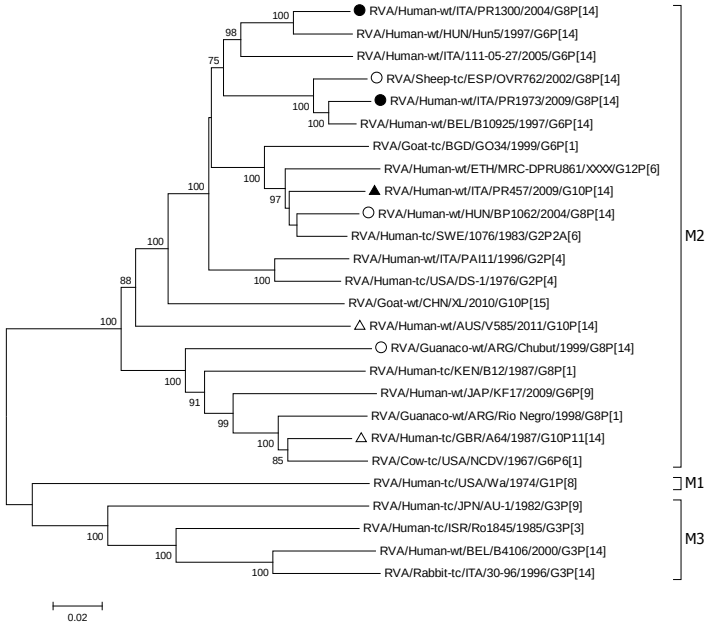

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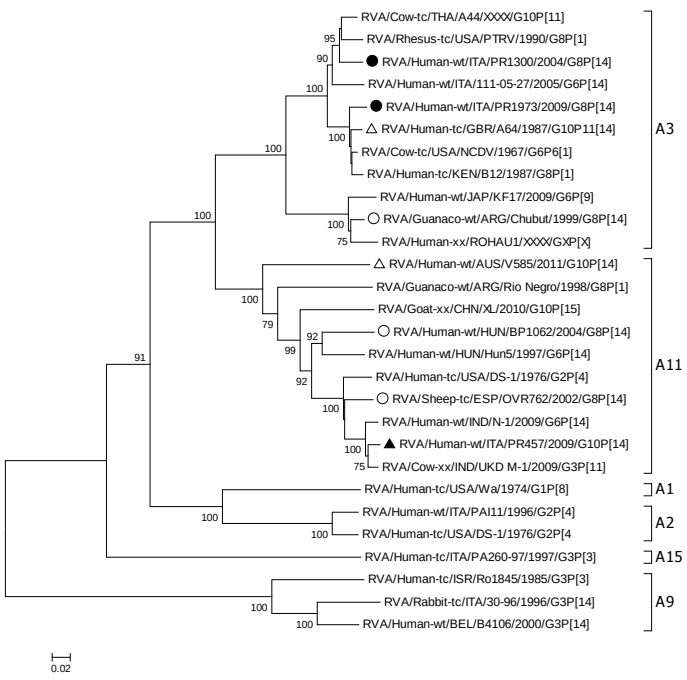

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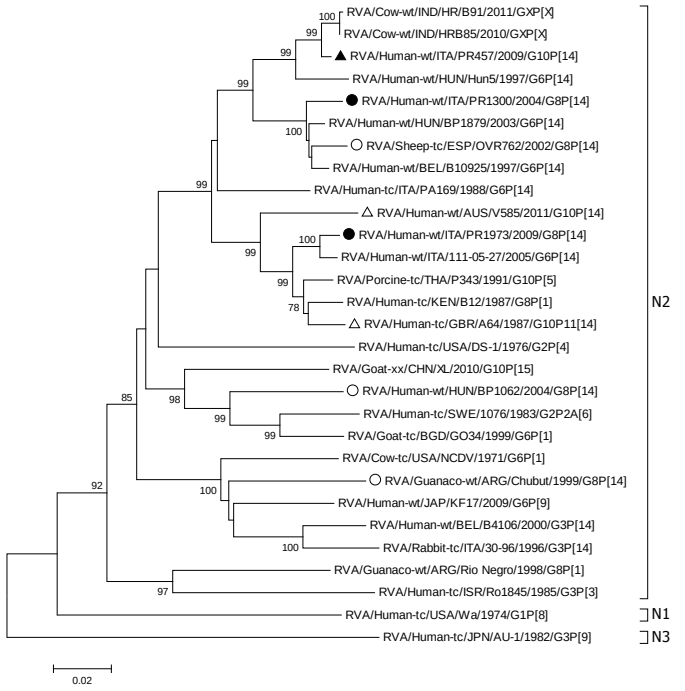

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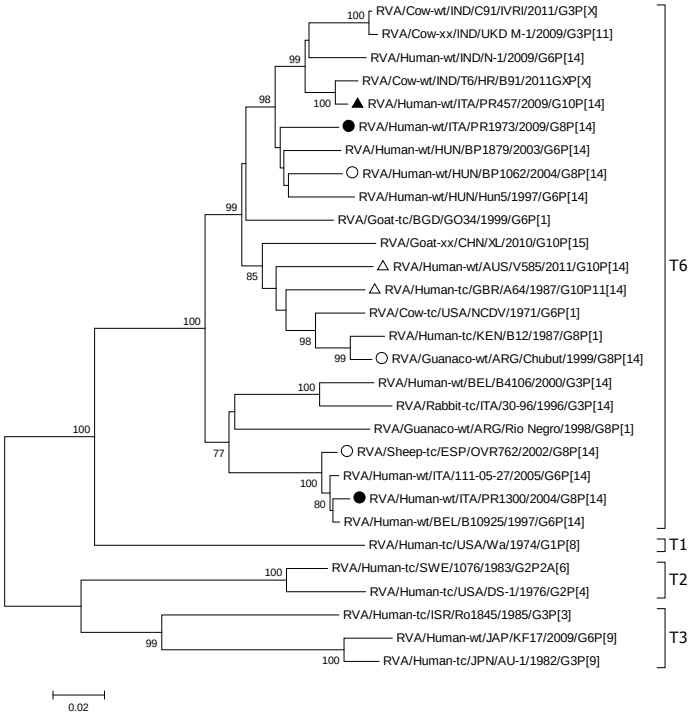

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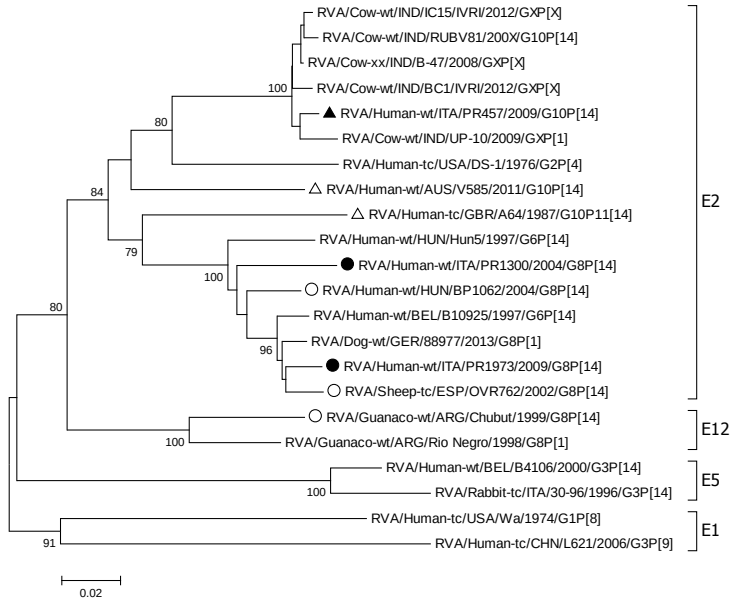

NSP5